

Effect of liquid crystals with cyclodextrin on the bioavailability of a poorly water-soluble compound, diosgenin, after its oral administration to rats.

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Abstract

Diosgenin, found in wild yam (*Dioscorea villosa*), has been shown to ameliorate diabetes and hyperlipidemia, increase cell proliferation in a human 3D skin model, and inhibit melanin production in B16 melanoma cells. It is also an active element in cosmeceutical and dietary supplements. Although the bioavailability of diosgenin is low due to its poor solubility and intestinal permeability, it was subsequently improved using a β -cyclodextrin (β -CD) inclusion complex. Recently liquid crystals (LCs) were shown to enhance the bioavailability of poorly water-soluble drugs. The purpose in the present study was to prepare diosgenin LCs and investigate the interaction between LC and β -CD in order to improve its bioavailability of diosgenin. Crystallinity and particle diameters of LCs in water were determined by small angle X-ray scattering (SAXS) and Zetasizer. Pharmacokinetic parameters were calculated using the plasma content of diosgenin after its oral administration to Wistar rats. Regarding the formation of glyceryl monooleate (GMO) and phytantriol (PHY) LC, SAXS patterns showed the hexagonal and cubic phases, respectively. Bioavailability was significantly enhanced after oral administration of LCs prepared by GMO than after diosgenin alone. The bioavailability was further improved with the combination of LC and β -CD than LC and water.

Keywords: Diosgenin, liquid crystal, glyceryl monooleate, phytantriol, β -cyclodextrin, oral administration.

1. Introduction

Many chemical compounds have recently been synthesized and characterized to develop new drug candidates in pharmaceutical industries. However, more than 40% of these compounds have been terminated due to poor dissolution and/or biomembrane permeability (Prentis et al., 1988; Venkatesh and Lipper, 2000). Poorly water-soluble compounds have been detected not only in medicines, but also in dietary and cosmetic supplements. Nevertheless, few studies have investigated the pharmacokinetics of these supplements. Thus, the pharmacokinetics of these supplements need to be investigated to improve their oral absorption. Diosgenin, which is extracted from wild yam (*Dioscorea villosa*) and fenugreek (*Trigonella foenum greaceum*), is a steroidal saponin (Taylor et al., 2000; Hooker, 2004). In our previous study, we demonstrated that the solubility of diosgenin in water and its bioavailability were poor (Okawara et al., 2010). The oral administration of diosgenin to diabetic rats significantly decreased plasma glucose levels (Pari et al., 2012). Furthermore, diosgenin decreased serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol levels in rats fed a high-fat diet (Gong et al., 2010). Diosgenin has been established as a raw material for the production of steroidal hormones in the pharmaceutical industry (Applezweig, 1969). It has also been used as dietary supplement in hormone replacement therapy for menopausal women (Russell et al., 2002; Benghuzzi et al., 2003). Orally administrated diosgenin was shown to improve skin thickness in ovariectomized mice, and enhanced DNA synthesis in a human 3D equivalent model (Tada et al., 2009). Furthermore, it inhibited melanogenesis in B16 melanoma cells by activating the phosphatidylinositol-3-kinase pathway (Lee et al., 2007). Based on these findings, diosgenin is considered as an active element in cosmeceutical and dietary supplements. We previously reported that the bioavailability of diosgenin was only 6% in rats. We have been

investigating ways by which to improve its low bioavailability. Our findings suggested that diosgenin and β -cyclodextrin (β -CD) formed 1:2 molar ratio inclusion complexes that improved the bioavailability of diosgenin to 45% in rats (Okawara et al., 2013). However, the β -CD inclusion complex has to be suspended in water when administered to rats, and it also takes time to prepare the complex.

Liquid crystals (LCs) are semisolids with crystalline structures combining the properties of both solid and liquid states (Yamada et al., 2011). Commonly encountered phases in LCs include the lamellar, bicontinuous cubic, and inverse hexagonal phases (Clogston et al., 2000). LCs are easily formed by various amphipathic lipids such as glyceryl monooleate (GMO) and phytantriol (PHY) in excess amounts of water (Lee et al., 2009; Costa-Balogh et al., 2010). Our previous report must be the first one for the LC using PHY (Yamada et al., 2011). Many studies reported that the oral administration of LC enhanced the bioavailability of poorly water-soluble drugs (Boyd et al., 2007; Nguyen et al., 2011; Lian et al., 2011). In this study, we prepare self-assembly LCs and dispersed LCs including diosgenin, and physicochemical measurements for LCs were performed using Zetasizer and small angle X-ray scattering (SAXS). LCs were administered to rats and their pharmacokinetic parameters were calculated for diosgenin. Furthermore, we elucidated the interaction between LC formation and β -CD solution for oral administration to rats.

2. Materials and methods

2.1. Materials

Diosgenin was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Polyoxyethylene hydrogenated castor oil 60 (HCO-60) was supplied from Nikko Chemicals (Tokyo, Japan). Sodium pentobarbital was obtained from Kyoritsu Seiyaku (Tokyo, Japan). GMO and PHY

were obtained from Tokyo Chemical Industry (Tokyo, Japan). β -CD, 6-methyl diosgenin, and other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Preparation of liquid crystals

LC was formed using GMO or PHY and an equal or greater volume of water, and involved a transition from the lamella phase to the hexagonal phase, and finally to the cubic phase by heating (Lee et al., 2009). To determine the solubility of diosgenin in lipids, each lipid was added to a tube with an excess amount of diosgenin. The mixture was heated at 37°C in a heating block to facilitate solubilization using a vortex mixer. Mixtures were shaken in a thermo-controlled incubator at 37°C for 2 h. After reaching equilibrium, each tube was centrifuged at 15,000 $\times g$ for 5 min and the supernatant was collected. After dilution, the content of diosgenin was determined using a liquid chromatography mass spectrometry (LCMS) system. Separation was achieved by an MXY01-01 (Michrom Biosources Inc., CA, U.S.A.) with a TSK gel ODS-100V column (2.0 \times 50 mm, 3 μ m) (TOSOH, Tokyo, Japan) at room temperature. The mobile phase consisted of methanol (90%) and H₂O (10%) containing 10 mM ammonium acetate. The flow rate was set to 150 μ L/min and detection was performed using an LCQ DECA XP^{Plus} mass spectrometer (Thermo Fisher Scientific Inc., MA, U.S.A.). Self-assembly LCs were prepared by dissolving diosgenin in each lipid at 5 mg/mL by heating at 70°C.

2.3. SAXS measurement

Equal volumes of water and self-assembly LC were mixed prior to measurements being taken. These samples were heated at 70°C, and mixed to homogenization using a vortex mixer. SAXS measurements were performed on a NANO-Viewer (Rigaku) with PILATUS 100K/RL

2D detector. The X-ray source was Cu K α radiation, wavelength 1.54 Å, operating at 45 kV and 110 mA. The sample-to-detector distance was chosen to be 375 mm. Each sample was placed between polyether ether ketone membranes and exposed for 10 min.

2.4. Dispersibility of LC

Dispersed LC was prepared by adding 10-fold of water to self-assembly LC. The mixture was heated on a heating block at 70°C, and shaken using a vortex mixer. These samples were centrifuged at 15,000 \times g for 5 min, and the water phase was collected. Samples were diluted 1000-fold in water prior to measurements being taken. Particle diameters and dispersion were measured by dynamic light scattering measurements on a Nano-ZS ZEN 3600 Zetasizer (Malvern, Worcestershire, UK) with water.

2.5. Solubility study

In the solubility study, 10-fold of water or 4 mM β -CD solution were added to self-assembly LC, and mixed at 37°C for 5 days. These samples were centrifuged at 15,000 \times g for 5 min, and the water phases were collected. The contents of diosgenin and each lipid were determined using an LCMS system after filtration with a 0.2 μ m membrane filter (ADVANTEC, Tokyo, Japan) and dilution.

2.6. Animals

Male Wistar rats (200 to 250 g) were provided from Japan SLC (Hamamatsu, Shizuoka, Japan). Animals were housed under a 12 h light and dark cycle in a temperature-controlled room (23 \pm 2°C). They had free access to food and water. The animal care protocol was approved by the Animal Care and Use Committee of Josai University (Saitama, Japan).

2.7. Pharmacokinetic studies

Intravenous and oral administration studies were performed to compare the pharmacokinetic parameters of diosgenin and its LC. Rats were fasted from at least 12 h prior dosing to 4 h after dosing. In the intravenous administration protocol, diosgenin was dissolved in saline containing 1% HCO-60, and 121 $\mu\text{g/kg}$ (body weight) of diosgenin was injected into the tail vein. For oral administration, a diosgenin suspension of 5 mg/mL or self-assembly LC formulation containing an equivalent amount of diosgenin was prepared and administered at a dose of 2 mL/kg (body weight). Diosgenin was suspended in saline containing 1% HCO-60. Self-assembly LC was prepared by dissolving diosgenin in PHY or GMO. In the LC group, self-assembly LC and equal amounts of water or β -CD solution (24 mM) were simultaneously administrated. Blood was collected from the tail vein into heparinized tubes at times ranging from 0 to 96 h, and was immediately separated by centrifugation. In a previous study, skin levels of diosgenin peaked 6 h after its oral administration (Okawara et al., 2013). Skin samples were taken from the entire abdomen 6 h after the oral administration. Each sample was stored at -30°C until analyzed.

2.8. Analytical procedure

6-methyl diosgenin was used as an internal standard to assess diosgenin levels in plasma and skin samples. Samples were added to methanol and extraction was achieved by sonication for 20 min at 37°C . These samples were centrifuged at $15,000 \times g$ for 5 min and the supernatants were collected. The content of diosgenin was determined by an LCMS system with a Tosoh TSK gel ODS-100V column (2.0×50 mm, 3 μm) at room temperature.

2.9. Pharmacokinetics and statistical analysis

Pharmacokinetics analysis was performed with nonlinear least-squares fitting. The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule. The absolute bioavailability was determined as AUC_{po}/AUC_{iv} , using mean AUC values for oral and intravenous doses. Tukey's multiple comparison tests was used to assess the significance of differences between groups. A p value of less than 0.05 was considered significant.

3. Results

3.1. SAXS measurements

The phase behavior of LCs made from GMO or PHY was confirmed by SAXS measurements. Representatives of the SAXS profiles of LCs are shown in Figure 1. SAXS curves revealed the presence of a hexagonal phase (with reflections spaced at $\sqrt{1}$, $\sqrt{3}$, and $\sqrt{4}$; Fig. 1A) for GMO LC and bicontinuous cubic phase (reflections at $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, $\sqrt{9}$, $\sqrt{10}$, and $\sqrt{12}$; Fig. 1B) for PHY LC. The phase behavior of LCs was not affected by the presence of diosgenin (see the lines a and b). These results confirmed that these lipids formed LCs when mixed with water at room temperature.

3.2 Dispersibility of LC

The particle diameter and dispersion of LCs were measured to confirm their conditions in the gastric and intestinal tract. The particle diameters of LCs made from GMO and PHY are listed in Table 1. Each particle diameter of LCs was nearly 100-200 nm and its distribution in water remained unchanged in the presence of diosgenin. PHY LC showed larger diameter than GMO LC both with and without diosgenin. Although the diameter of a particle of PHY became small by diosgenin, it is unknown for details.

3.3. Solubility study

The solubility of diosgenin and lipids in water or β -CD solution are listed in Table 2. The solubility of diosgenin was increased by β -CD and GMO, whereas it was decreased by PHY. It is suggested that the lower release of diosgenin was caused by PHY melting point (70°C). More diosgenin was released from LCs in β -CD solution than in water. Correspondingly, the solubilities of GMO and PHY in water were increased by β -CD.

3.4. Pharmacokinetics of diosgenin self-assembly LC with CD solution after oral administration

Fig. 2 shows the mean plasma diosgenin concentration-time curves after oral administration of self-assembly LC and CD solution in Wistar rats. Table 3 shows the calculated pharmacokinetics parameters. The maximum plasma concentration of diosgenin (C_{max}) after oral administration of GMO LC was higher than of the diosgenin suspension. The time to reach the maximum plasma concentration (T_{max}) of GMO LC was similar to that of the suspension (Fig. 2A). However, the T_{max} was significantly higher and C_{max} was significantly lower with PHY LC than with the diosgenin suspension (Fig. 2B). Plasma concentrations were higher in the β -CD combination groups than in self-assembly LC with water. Diosgenin bioavailability was significantly higher after the oral administration of GMO LC with β -CD solution than after GMO LC with water.

3.5. Skin distribution of diosgenin

The distributions of diosgenin in skin were determined after the oral administration of diosgenin or its LC and summarized in Figure 3. The contents of diosgenin in skin 6 h after its administration alone, with β -CD solution, GMO LC, GMO LC with β -CD solution, PHY LC, and PHY LC with β -CD solution were 23.95, 90.70, 178.21, 371.79, 11.39, and 8.93 ng/g skin, respectively. The content of diosgenin was significantly higher in the GMO LC with β -CD solution groups than in the diosgenin suspension.

4. Discussion

We initially prepared diosgenin LCs and characterized their physical properties. Self-assembly LCs and dispersed LCs were prepared and analyzed using SAXS and Zetasizer. The SAXS data demonstrated that GMO and PHY, which dissolved diosgenin, formed hexagonal and cubic phases, respectively, when mixed with equal volumes of water at room temperature. The particle diameter of LCs was nearly 100-200 nm in water. These results suggested that GMO and PHY LCs are dispersed in the gastrointestinal tract as microdroplets (Jin et al., 2013). The solubility of diosgenin was increased by β -CD and GMO. Further enhancements in its solubility were obtained when LCs were combined with β -CD solution. These results indicated that β -CD solution enhanced the release of diosgenin and lipids from LCs.

Plasma diosgenin levels were relatively low after oral administration of the diosgenin suspension, and were similar to those in our previous study (Okawara et al., 2013). However, GMO LC significantly improved its bioavailability. Previous studies reported that GMO LC were strongly bioadhesive and stimulated entrapped-drug permeation through the intestinal mucosa (Geraghty et al., 1997; Lai et al., 2009). T_{max} was higher with PHY LC than with the diosgenin suspension. Nguyen et al. suggested that PHY LC retained in the gastrointestinal tract may slowly diffuses, while suppressing drug release; this is consistent with the results of the present study (Nguyen et al., 2010). Furthermore, the combination of self-assemble LC and β -CD solution enhanced the plasma concentration and bioavailability more than LC alone. Different patterns were confirmed in plasma diosgenin concentration–time curves after the oral administration of GMO LC and PHY LC. These may have been caused by differences in the melting points of lipids than changes in LC phases. The skin distribution of diosgenin was higher after the oral administration of GMO LC and GMO LC with β -CD solution than that of

the diosgenin suspension. A lower skin content was observed after the oral administration of PHY LC and PHY LC with β -CD solution. A previous study reported a correlation between plasma concentrations of diosgenin and skin content (Okawara et al., 2013). These findings suggested that PHY LC and PHY LC with β -CD solution maintained the skin content of diosgenin over 48 h. Although further research is needed, self-assembly LCs may control the release of drugs included in LC. Furthermore, the results of the present study indicate that β -CD solution improve the release of drugs from LCs.

Conclusions

The aim of this study was to improve the bioavailability of diosgenin. We prepared diosgenin LCs and evaluated their combined effect with β -CD solution. The solubility, bioavailability, and skin distribution of diosgenin were much better with LC in β -CD solution than that with LC alone. These results indicate that β -CD solution increases diosgenin and lipid release from LCs.

References

- Applezweig, N., 1969. Steroids. Chem. Week 17, 57-72.
- Benghuzzi, H., Tucci, M., Eckie, R., Hughes, J., 2003. The effects of sustained delivery of diosgenin on the adrenal gland of female rats. Biomed. Sci. Instrum. 39, 335-340.
- Boyd, B.J., Khoo, S.M., Whittaker, D.V., Davey, G., Porter, C.J., 2007. A lipid-based liquid crystalline matrix that provides sustained release and enhanced oral bioavailability for a model poorly water soluble drug in rats. Int. J. Pharm. 340, 52-60.
- Clogston, J., Rathman, J., Tomasko, D., Walker, H., Caffrey, M., 2000. Phase behavior of a monoacylglycerol: (myverol 18-99K)/water system. Chem. Phys. Lipids 107, 191-220.
- Costa-Balogh, F.O., Sparr, E., Sousa, J.J., Pais, A.C., 2010. Drug release from lipid liquid crystalline phases: relation with phase behavior. Drug Dev. Ind. Pharm. 36, 470-481.
- Geraghty, P.B., Attwood, D., Collett, J.H., Sharma, H., Dandiker, Y., 1997. An investigation of the parameters influencing the bioadhesive properties of Myverol 18-99/water gels. Biomaterials 18, 63-67.
- Gong, G., Qin, Y., Huang, W., Zhou, S., Wu, X., Yang, X., Zhao, Y., Li, D., 2010. Protective effects of diosgenin in the hyperlipidemic rat model and in human vascular endothelial cells against hydrogen peroxide-induced apoptosis. Chem. Biol. Interact. 184, 366-375.
- Hooker, E., 2004. Final report of the amended safety assessment of Dioscorea Villosa (Wild Yam) root extract. Int. J. Toxicol. 23 Suppl 2, 49-54.
- Jin, X., Zhang, Z.H., Sun, E., Tan, X.B., Li, S.L., Cheng, X.D., You, M., Jia, X.B., 2013. Enhanced oral absorption of 20(S)-protopanaxadiol by self-assembled liquid crystalline nanoparticles containing piperine: *in vitro* and *in vivo* studies. Int. J. Nanomedicine 8, 641-652.

276 Lai, J., Chen, J., Lu, Y., Sun, J., Hu, F., Yin, Z., Wu, W., 2009. Glyceryl monooleate/poloxamer
 277 407 cubic nanoparticles as oral drug delivery systems: I. *In vitro* evaluation and enhanced
 278 oral bioavailability of the poorly water-soluble drug simvastatin. AAPS PharmSciTech 10,
 279 960-966.

280 Lee, J., Jung, K., Kim, Y.S., Park, D., 2007. Diosgenin inhibits melanogenesis through the
 281 activation of phosphatidylinositol-3-kinase pathway (PI3K) signaling. Life Sci. 81,
 282 249-254.

283 Lee, K.W., Nguyen, T.H., Hanley, T., Boyd, B.J., 2009. Nanostructure of liquid crystalline
 284 matrix determines *in vitro* sustained release and *in vivo* oral absorption kinetics for
 285 hydrophilic model drugs. Int. J. Pharm. 365, 190-199.

286 Lian, R., Lu, Y., Qi, J., Tan, Y., Niu, M., Guan, P., Hu, F., Wu, W., 2011. Silymarin glyceryl
 287 monooleate/poloxamer 407 liquid crystalline matrices: physical characterization and
 288 enhanced oral bioavailability. AAPS PharmSciTech 12, 1234-1240.

289 Nguyen, T.H., Hanley, T., Porter, C.J., Boyd, B.J., 2011. Nanostructured liquid crystalline
 290 particles provide long duration sustained-release effect for a poorly water soluble drug after
 291 oral administration. J. Control. Release 153, 180-186.

292 Nguyen, T.H., Hanley, T., Porter, C.J., Larson, I., Boyd, B.J., 2010. Phytantriol and glyceryl
 293 monooleate cubic liquid crystalline phases as sustained-release oral drug delivery systems
 294 for poorly water-soluble drugs II. *In-vivo* evaluation. J. Pharm. Pharmacol. 62, 856-865.

295 Okawara, M., Tokudome, Y., Todo, H., Sugibayashi, K., Hashimoto, F., 2010. Diosgenin
 296 disposition in rats after i.v. and p.o. administration. J. Pharm. Sci. Technol. Jpn. 70, 82-86.

297 Okawara, M., Tokudome, Y., Todo, H., Sugibayashi, K., Hashimoto, F., 2013. Enhancement of
 298 diosgenin distribution in the skin by cyclodextrin complexation following oral
 299 administration. Biol. Pharm. Bull. 36, 36-40.

300 Pari, L., Monisha, P., Mohamed Jalaludeen, A., 2012. Beneficial role of diosgenin on oxidative
 301 stress in aorta of streptozotocin induced diabetic rats. *Eur. J. Pharmacol.* 691, 143-150.
 302 Prentis, R.A., Lis, Y., Walker, S.R., 1988. Pharmaceutical innovation by the seven UK-owned
 303 pharmaceutical companies (1964-1985). *Br. J. Clin. Pharmacol.* 25, 387-396.
 304 Russell, L., Hicks, G.S., Low, A.K., Shepherd, J.M., Brown, C.A., 2002. Phytoestrogens: a
 305 viable option? *Am. J. Med. Sci.* 324, 185-188.
 306 Tada, Y., Kanda, N., Haratake, A., Tobiishi, M., Uchiwa, H., Watanabe, S., 2009. Novel effects
 307 of diosgenin on skin aging. *Steroids* 74, 504-511.
 308 Taylor, W.G., Elder, J.L., Chang, P.R., Richards, K.W., 2000. Microdetermination of diosgenin
 309 from fenugreek (*Trigonella foenum-graecum*) seeds. *J. Agric. Food Chem.* 48, 5206-5210.
 310 Venkatesh, S., Lipper, R.A., 2000. Role of the development scientist in compound lead
 311 selection and optimization. *J. Pharm. Sci.* 89, 145-154.
 312 Yamada, K., Yamashita, J., Todo, H., Miyamoto, K., Hashimoto, S., Tokudome, Y., Hashimoto,
 313 F., Sugibayashi, K., 2011. Preparation and evaluation of liquid-crystal formulations with
 314 skin-permeation-enhancing abilities for entrapped drugs. *J. Oleo Sci.* 60, 31-40.

Figure legends

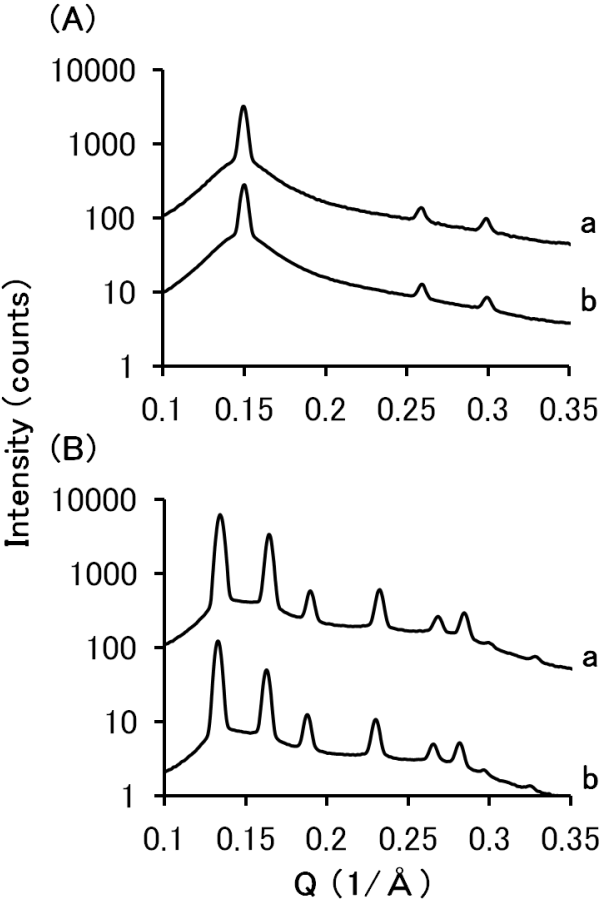
Figure 1. SAXS profiles for GMO (A) and PHY (B). The profiles are shown for LC with diosgenin (a) and without diosgenin (b). LCs were prepared by mixing equal volumes of self-assembly LC and water at 70°C.

Figure 2. Plasma profiles after the oral administration of diosgenin: A- (○), diosgenin suspension; (□), GMO self-assembly LC with water; (■), GMO self-assembly LC with β-CD solution; B-(○), diosgenin suspension; (□), PHY self-assembly LC with water; and (▲), PHY self-assembly LC with β-CD solution. Each point shows the mean ± S.E. of 3 to 8 experiments. *: $p < 0.05$ significantly different from the diosgenin suspension. †: $P < 0.05$ significantly different from its self-assembly LC (Tukey's test).

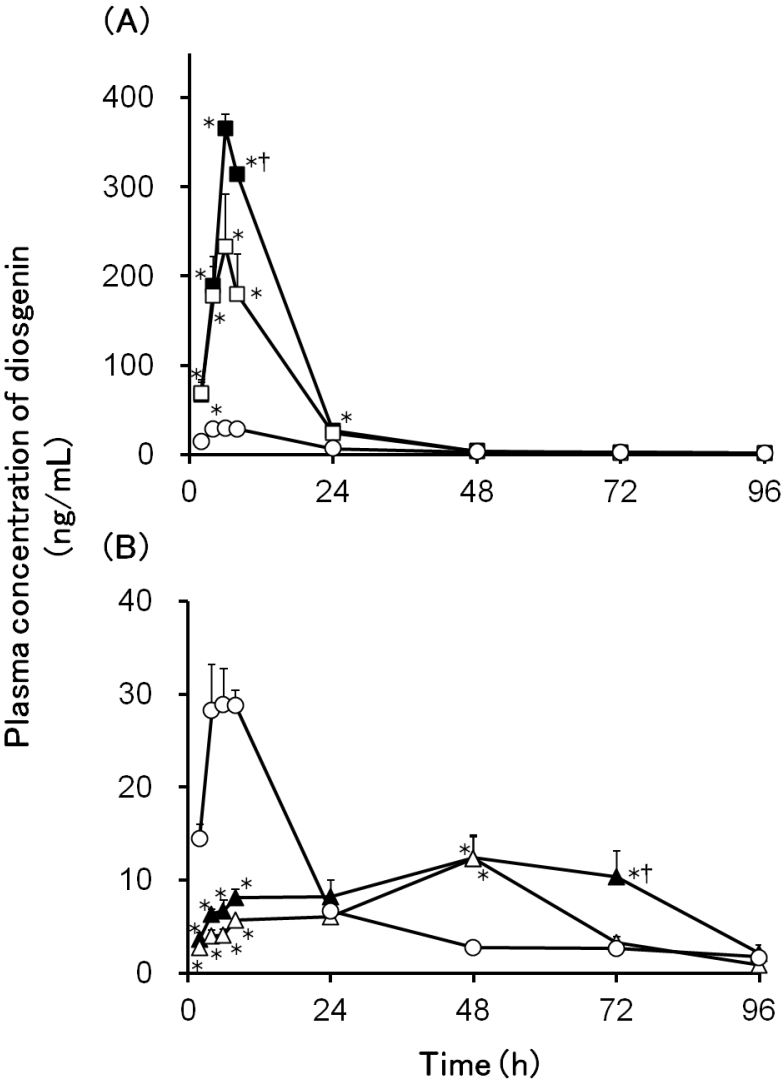
Figure 3. Skin distribution of diosgenin after oral doses of diosgenin and LC. Skin samples were collected 6h after the oral administration of diosgenin and LC. Each column shows the mean ± S.E. of 3 to 4 experiments. *: $p < 0.05$ significantly different from the diosgenin suspension (Tukey's test).

331 **Figures**

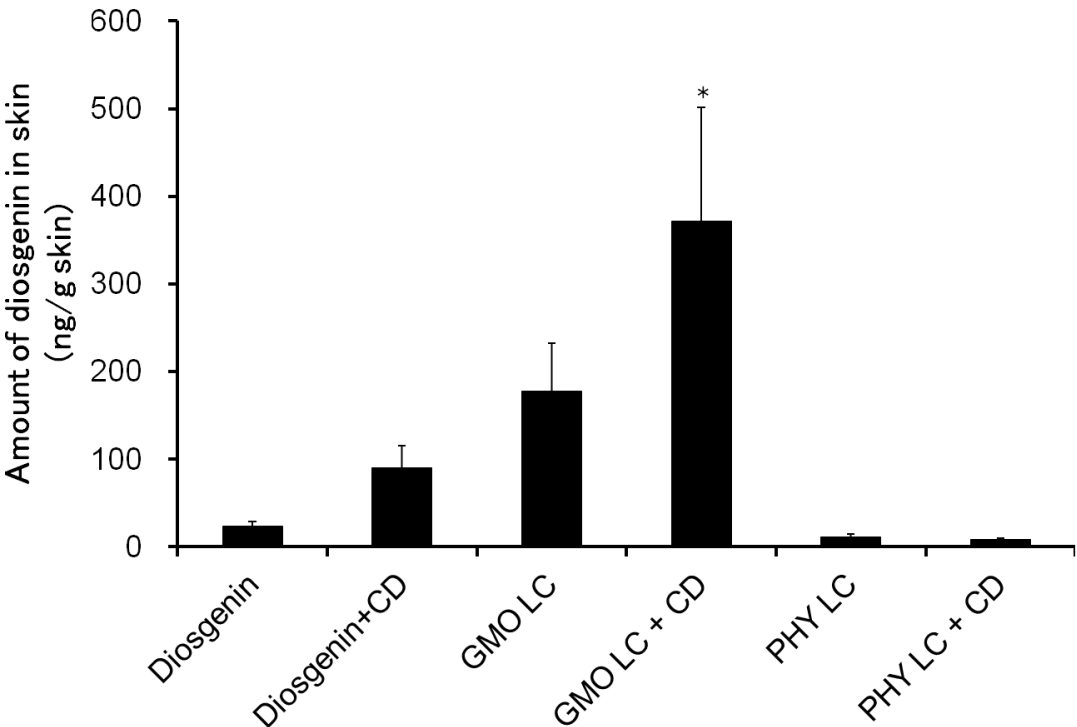
332 Fig. 1



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336 Fig. 3



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Tables

Table 1. Particle diameter of LCs.

	Particle diameter (nm)	
	Without diosgenin	With diosgenin
GMO	112.1 ± 0.5	117.6 ± 0.5
PHY	212.1 ± 0.8	138.1 ± 0.5

Self-assembly LCs were dispersed in water at 37°C. The particle diameter of each formation was measured after centrifugation and dilution.

Table 2. Solubility of diosgenin and lipids in water or β -CD solution.

	Solubility of diosgenin			Solubility of lipids		
	in water (ng/mL)		CD/Water ratio	in water (μ g/mL)		CD/Water ratio
	Water	CD solution		Water	CD solution	
GMO	2,210 ± 460	8,360 ± 400	3.77	409 ± 40	464 ± 112	1.14
PHY	4.50 ± 1.11	3,550 ± 220	789	3.99 ± 0.48	16.9 ± 1.9	4.24
Without lipids	10.5 ± 3.5	3,260 ± 170	310	-	-	-

Diosgenin was dissolved in GMO or PHY at 5 mg/mL. A ten-fold amount of water or 4 mM β -CD solution was added, and mixed at 37°C for 5 days. Each value shows the mean ± S.E. of 3 experiments.

Table 3. Pharmacokinetic parameters after the oral administration of the diosgenin suspension and self-assembly LCs with water or CD solution.

	<i>AUC_{po}</i> (ng · h/mL)	<i>C_{max}</i> (ng/mL)	<i>T_{max}</i> (h)	Bioavailability (%)	Enhancement ratio
GMO LC + CD	4403 ± 132*†	239 ± 2*	5.6 ± 0.2	47.1 ± 1.4*†	6.2
GMO LC	2934 ± 465*	164 ± 36*	6.0 ± 1.2	31.4 ± 5.0*	4.1
PHY LC + CD	1000 ± 191	11.4 ± 1.7*	22 ± 3*	10.2 ± 1.8	1.4
PHY LC	633.9 ± 62.0	8.38 ± 0.52*	22 ± 4*	7.30 ± 0.05	0.9
Diosgenin	711.4 ± 53.3	23.0 ± 1.9	5.0 ± 0.7	7.61 ± 0.51	1.0

Each value shows the mean ± S.E. of 3 to 4 experiments. *: p<0.05 significantly different from diosgenin. †: p<0.05 significantly different from its LC. (Tukey's test).